

Near-infrared reflectance spectroscopy prediction of enzyme-released glucose in alfalfa stems

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Alfalfa (*Medicago sativa* L.) has a high potential for sustainable bioethanol production, particularly because of its low reliance on N fertilizer. Genetic improvement for the accumulation of readily fermentable non-structural carbohydrates (NSC) and the saccharification of structural carbohydrate (SC) could significantly increase ethanol conversion rate. Genetic gains for these traits are tributary to the availability of screening techniques for the precise identification of superior genotypes with increased potential for the production of fermentable carbohydrates.

When assessing the genetic variability of parameters linked to cellulosic ethanol production (concentrations of non-structural and structural sugars), our results showed that a large genetic variability exists within and among winterhardy- and biomass-type alfalfa cultivars. We also developed an efficient enzymatic assay to measure alfalfa stem degradability, based on the quantity of glucose released by a customized commercially available enzymatic cocktail. Again, we observed a large genetic diversity for this trait in six different genetic backgrounds. Despite its robustness, this test is labour intensive, thus limiting analytical capabilities. Here, we assessed near-infrared reflectance spectroscopy (NIRS) as a high-throughput technique to predict cell wall (CW) degradability in a large number of lignified alfalfa stem samples.

NIRS was successfully applied to accurately predict CW degradability in four different alfalfa cultivars ($R^2=0.94$), measured by glucose released following enzymatic saccharification. NIRS predictions showed that there was a large genetic diversity for enzyme-released glucose. The 10 genotypes with the highest (D+) and the lowest (D-) amounts of enzyme-released glucose of a biomass-type (Orca) and a winterhardy-type (54V54) cultivar were further characterized. D+ genotypes were at least 35% more degradable than D- genotypes. Determination of CW composition by chemical analyses showed that a higher lignin content of the D- genotypes was closely related to their lower enzyme-released glucose ($R=-0.83$).

For each cultivar tested, 20 D+ and 20 D- genotypes were intercrossed to generate D+ and D- F_1 populations. Assessment of CW enzyme-released glucose in the progenies showed that this trait is genetically inherited. Through recurrent selection, we were able to exploit this heterogeneity of enzyme-released glucose to produce a new germplasm more prone to enzymatic saccharification. Assessment of CW degradability (enzyme-released glucose) of progenies, after one cycle of divergent selection, clearly demonstrated the possibility to create a selection program based on this trait. Progenies with contrasting CW degradability characteristics are currently being used to develop molecular markers for this trait. The large genetic diversity of enzyme-released glucose and its potential for selection support the huge potential of alfalfa in the sustainable production of bio-ethanol.